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GENETIC STRUCTURE OF GENE RESERVE POPULATION OF WHITEBACKED CATTLE BASED ON BLOOD GROUP POLYMORPHISM*

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Abstract

Antigenic composition of blood was determined in 92 Whitebacked cattle from north-eastern Poland using 75 standardized test reagents. Frequency of phenogroups, degree of heterozygosity and effective number of alleles were calculated in the A, B, C and S blood group systems. Twenty-nine phenogroups were identified in the B system, in which the highest frequency was found for I2Q" (0.1849), G2Y2E'1Q'D" (0.1631), E'3G"1Q" (0.0978) and Q" (0.0978). In this system, the degree of homozygosity was 9.14% and the effective number of alleles was 10.9. Analysis of genotype distribution in the F and R' systems showed significant differences between the expected and observed number of genotypes, indicating a disturbed genetic balance. Compared to previous studies on genetic structure of other breeds included in the genetic resources conservation programme, Whitebacked cattle had a higher degree of homozygosity and a much lower number of phenogroups in the B and C systems, which points to lower genetic variation in the analysed population of Whitebacked cattle.

Key words: cattle, blood groups, genetic structure

In recent years, increasing attention has been given to the determination and preservation of biological diversity. The FAO's Global Plan of Action for Animal Genetic Resources recommends that certain breeds and varieties of farm animals should be protected due to their economic, scientific and cultural importance (Hammond, 1997). Efforts at preserving biodiversity of different breeds should also include comprehensive analysis of their genetic structure, performed using the largest possible number of genetic markers.

Long-term research has demonstrated that blood groups in cattle vary widely. To date, about 100 antigenic factors, determined by genes located in 12 chromosome pairs, have been detected in this species (ISAG Comparison Test Cattle 2003/2004).

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The large number of antigens transmitted in different combinations (especially in complex B and C systems) as well as their simple inheritance pattern provide considerable scope for their use in research on genetic characterization of different breeds and varieties (Trela et al., 1982; Gonzalez et al., 1987; Ertugrul and Alpan, 1990; Mortari, 1990; Barker et al., 1997; Rychlik et al., 1999; Duniec et al., 2002 b) and in evaluation of changes that occur in the genetic structure of cattle populations being improved (Rychlik, 1986; Kantanen et al., 1999). This type of research is of special importance for native breeds threatened with extinction because they are one of the elements needed to compile an inventory of livestock genetic resources. In Poland, the cattle genetic resources conservation programme covers four breeds: Polish Red, the oldest breed that has been unofficially protected since 1973; Whitebacked (under conservation since 2003); Polish Red-and-White (under conservation since 2007); and Polish Black-and-White (conserved since 2008). Three of these breeds have been analysed for genetic structure based on blood groups: Polish Red (Trela et al., 1984; Rychlik et al., 1999), Polish Black-and-White (Trela et al., 1982; Rychlik, 1986) and Polish Red-and-White (Trela, 1977; Rychlik et al., 2008). The objective of the present study was to perform extensive genetic characterization of a small population of Whitebacked cattle from north-eastern Poland based on all known blood group systems.

Material and methods

Ninety-two samples of blood obtained in 2005–2009 from Whitebacked cattle in north-eastern Poland were investigated. Antigenic characters in 12 blood group systems (A, B, C, F, J, L, M, S, Z, N', R', T') were determined by the haemolytic test using 75 test sera obtained at the Department of Animal Immuno- and Cytogenetics of the National Research Institute of Animal Production and checked by international comparison tests organized by the International Society for Animal Genetics (ISAG).

The genotypes of the animals were determined by analysing antigenic composition of blood in offspring and parents. The genotypes determined in the A, B, C and S systems were used to calculate frequency of phenogroups. A similar procedure was employed to calculate frequency of genes determining antigenic characters in the F and R' systems, in which the genotype of the animal studied can be determined based on phenotype. In the J, L, M, Z, N' and T' systems with one dominant allele and one recessive allele, gene frequency was calculated using the formula:

$$P = 1 - \sqrt{R}$$

where:
P – frequency of a gene controlling an antigenic character,
R – frequency of recessive animals.

In complex systems (A, B, C, S), the degree of homozygosity was calculated to express the sum of squares of all phenogroups and the effective number of alleles at a locus (Kimura and Crow, 1964).

The state of genetic equilibrium for the investigated population of cattle was determined by calculating the expected and observed distributions of genotypes in the F and R' systems.

Results

In the 12 blood group systems in the investigated population of Whitebacked cattle, a total of 73 erythrocyte antigens were identified: A1, A2, H, PLB-4, D, PLB-8, B, G1, G2, G3, I1, I2, K, O1, O2, O3, O4, P1, P2, Y1, Y2, A'1, A'2, A'3, D', E'1, E'2, E'3, G', I'1, I'2, J', K', O', P', Q', Y', A"2, D", G"1, G"2, O", Q", C1, C2, E, R1, R2, W, X1, X2, C', L', C", PLB-9, F, V, J, L, M, S, H', U1, U2, U'1, U'2, H", U", Z, N', R', S', T'.

In the complex systems, they formed phenogroups inherited as complexes. The frequency of phenogroups from the A, B, C and S blood group systems is presented in Table 1, which also provides the degree of homozygosity and the effective number of alleles calculated for these systems.

The degree of homozygosity was 28.81% for the A system, 9.14% for the B system, 10.72% for the C system and 26.78% for the S system. The effective number of alleles in the above systems was 3.5, 10.9, 9.3 and 3.7, respectively.

Table 1. Frequency of phenogroups in A, B, C and S systems in the investigated population of Whitebacked cattle

System	Phenogroups	Frequency
1	2	3
EAA	A1	0.1522
	A1DHPLB-4	0.0217
	A1DPLB-4	0.0109
	A1H	0.0761
	A2H	0.0054
	A2DPLB-4	0.0652
	DPLB-4	0.0163
	DPLB-8	0.4783
	HDHPLB-4	0.0163
	-	0.1576
Degree of homozygosity (%)		**28.81**
Effective number of alleles		**3.47**

System	Phenogroups	Frequency
1	2	3
EAC	C1	0.0109
	C1E	0.1359
	C1ER1	0.0326
	C1EW	0.0163
	C1EX2	0.0054
	C1EL'	0.0109
	C1R2W	0.0054
	C1R2X2L'	0.0109
	C1WX2	0.0544
	C2R2WX2PLB-9	0.0054
	C2R2WC'PLB-9	0.0380
	EC"	0.2283
	R1WX2C"	0.0109
	R2WX2C"	0.0217

Table 1 – contd.

1	2	3
EAB	BG2KO4Y2A'1I'2O'A"2D"O"Q"	0.0435
	BG2KO4A'1E'3G'A"2D"G"1Q"	0.0380
	BI1Q"	0.0163
	BO1Y2D'I'1Q"	0.0326
	BO1I'2Q"	0.0109
	BO3Y1A'1E'3G'I'2P'Q'G"1	0.0326
	G2I1A"2D"Q"	0.0054
	G2O1A"2D"Q"	0.0054
	G2Y2E'1Q'D"	0.1631
	G3O1T1A'3E'3I'2K'G"2Q"	0.0109
	I1O2A'3J'K'O'O"Q"	0.0109
	I1Q'	0.0163
	I1Q"	0.0054
	I2Q"	0.1849
	O1A'1I'2Q"	0.0326
	O1A'2I'1Q"	0.0054
	O2A'3J'K'O'O"Q"	0.0109
	O4Y2A'1Q"	0.0326
	O4Y2D'E'1I'2O'G"2O"Q"	0.0109
	O4D'E'3G'O'G"2O"Q"	0.0109
	O4O'O"Q"	0.0326
	P1I'1Q"	0.0217
	Y1E'3G'Y'G"1Q"	0.0109
	E'1Q"	0.0109
	E'3G"1Q"	0.0978
	I'1Q'	0.0217
	I'1Q"	0.0054
	I'2Q'	0.0217
	Q"	0.0978
Degree of homozygosity (%)		**9.14**
Effective number of alleles		**10.9**
	R2X2C"	0.0652
	R2C'C"	0.0109
	R2C"	0.0326
	WX2C"	0.0109
	WL'C"	0.0163
	WC"	0.0109
	X1C"	0.0326
	X2L'C"	0.0109
	X2C"	0.0761
	C'L'C"	0.0054
	C'C"	0.0054
	L'C"	0.0054
	C"	0.1304
Degree of homozygosity (%)		**10.72**
Effective number of alleles		**9.33**
EAS	SH'	0.3261
	U1H'H"	0.0869
	U1H'	0.0054
	U1H'H"U"	0.0109
	H'	0.3587
	U'1	0.0435
	U'2	0.0109
	H"	0.0054
	-	0.1522
Degree of homozygosity (%)		**26.78**
Effective number of alleles		**3.73**

Table 2. Frequency of genes in F, J, L, M, Z, N', R' and T' blood group systems in the investigated population of Whitebacked cattle

System	Gene	Frequency	System	Gene	Frequency
EAF	F	0.9293	EAZ	Z	0.1725
	V	0.0707		z	0.8275
EAJ	J	0.1665	EAN'	N'	0.0792
	j	0.8335		n'	0.9208
EAL	L	0.1992	EAR'	R'	0.0978
	l	0.8008		S'	0.9022
EAM	M	0.0388	EAT'	T'	0.0502
	m	0.9612		t'	0.9498

Table 2 gives the frequency of antigenic characters and genes in the F, J, L, M, Z, N', R' and T' systems.

Table 3. Observed and expected distributions of genotypes in F and R' systems

System	Genotype	Observed	Expected	Chi^2
F	F/F	80	77.6	
	F/V	9	13.8	11.34**
	V/V	3	0.6	
R'	R'/R'	4	0.9	
	R'/S'	10	16.2	13.16**
	S'/S'	78	74.9	

**P<0.01.

Genetic equilibrium in the investigated population of cattle was tested based on the F and R' systems (Table 3). The significance of differences, calculated using chi-square test between the expected and observed number of genotypes showed the lack of genetic equilibrium in the Whitebacked cattle population under study.

Discussion

In addition to microsatellite DNA polymorphism, blood groups are a perfect tool for studying the genetic structure of animals, which in recent years has been investigated mainly in terms of evaluation of biodiversity. For biodiversity to be preserved, it is necessary to predict, prevent and eliminate the underlying reasons behind the erosion of biodiversity.

The genetic structure of the investigated population of Whitebacked cattle was characterized based on frequency analysis of genes controlling erythrocyte antigens in 12 blood group systems. Guided by results of previous studies, we paid special attention to the complex systems (A, B, C, S) that provide more information on genetic variation compared to simple systems (Rychlik et al., 1999; Duniec et al., 2001; Duniec et al., 2002 a). In the A blood group system of the cattle studied, there were 6 erythrocyte antigens: A1, A2, H, D, PLB-4 and PLB-8. They formed 10 phenogroups (Table 1), the most and least frequent of which were DPLB-8 (0.4783) and A1DPLB-4 (0.0109), respectively. A high frequency of D-PLB8 was also found in an earlier study with the A blood group system in Black-and-White (Duniec et al., 2001), Polish Red (Duniec et al., 2001; Rychlik et al., 1999) and Red-and-White cattle (Rychlik et al., 2008). No Z' antigen was found in the population studied. The degree of homozygosity, calculated in this system, was 28.81% with the effective number of alleles of 3.5. These values were similar to those reported for Polish Red cattle (Rychlik et al., 1999), in which they were 18.48% and 5.4, respectively.

In the B system we determined the most (37) antigenic characters (B, G1, G2, G3, I1, I2, K, O1, O2, O3, O4, P1, P2, Y1, Y2, A'1, A'2, A'3, D', E'1, E'2, E'3, G',

I'1, I'2, J', K', O', P', Q', Y', A"2, D", G"1, G"2, O", Q"), which were transmitted in different combinations to form 29 B-phenogroups (Table 1). The most frequent phenogroups in this system were I2Q" (0.1849), G2Y2E'1Q'D" (0.1631), E'3G"1Q" (0.0978) and Q" (0.0978).

These B-phenogroups were also reported to be the most frequent in Black-and-White cattle (Duniec et al., 2002 a) and were characteristic of this breed in earlier studies (Trela et al., 1982; Rychlik, 1986). The degree of homozygosity and the effective number of alleles, calculated for the investigated population of Whitebacked cattle was 9.14% and 10.9, respectively. These values were much higher than those reported recently for Polish Red cattle (6.36% and 15.7, respectively) (Rychlik et al., 1999) and higher than those found in Red-and-White cattle (6.01% and 16.65, respectively) (Rychlik et al., 2008). Also in earlier studies with Black-and-White cattle (Trela et al., 1982; Rychlik, 1986), the degree of homozygosity was lower (about 8.5%). In all the recent studies cited above, the number of B-phenogroups was much higher than in Whitebacked cattle. The number of B-phenogroups identified was 77 for Polish Red (Rychlik et al., 1999), 72 for Red-and-White (Rychlik et al., 2008) and 60 in the latest study on Black-and-White cattle (Duniec et al., 2002 a). The relatively high degree of homozygosity and the small number of B-phenogroups in Whitebacked cattle indicate that genetic variation in the discussed blood group system is much lower than in other breeds included in the genetic resources conservation programme.

In the C system, we identified 12 antigenic characters (C1, C2, E, R1, R2, W, X1, X2, C', L', C", PLB-9), which formed 27 phenogroups (Table 1). The highest frequencies were found for EC" (0.2283), C1E (0.1359), C" (0.1304), X2C" (0.0761) R2X2C" (0.0652) and C1WX2 (0.0544), and the lowest for C1EX2, C1R2W, C2R2WX2PLB-9, C'L'C", C'C" and L'C" phenogroups (0.0054). The high frequency of C1E (0.176) and C1WX2 phenogroups (0.160) was also reported in an earlier study concerning the C system in Black-and-White cattle (Duniec et al., 1981). In this system, the degree of homozygosity was 10.72% and the effective number of alleles was 9.33. Greater diversity in this system was found for Polish Red (Rychlik et al., 1999) and Red-and-White cattle (Rychlik et al., 2008), in which the degree of homozygosity and the effective number of C-phenogroups were 4.81%, 20.7 and 6.01%, 16.65, respectively. A much greater number of phenogroups was also found in these breeds.

In the S blood group system, we identified 9 phenogroups (Table 1), in which 8 antigenic characters were determined (S, U1, U2, H', U'1, U'2, H", U") and the most frequent phenogroups were H' (0.3587), SH' (0.3261) and S- (0.1532).

The degree of homozygosity and the effective number of alleles were 26.78% and 3.7, respectively. A study on the inheritance of antigenic characters in cattle in the S blood group system (Rychlik and Duniec, 2005) showed that the degree of homozygosity (20.41% in Black-and-White, 34.5% in Red-and-White and 24.7% in Polish Red cattle) was lower than in the Whitebacked population under study except the Red-and-White breed. Also the number of phenogroups in this system was lower in Whitebacked cattle than in Black-and-White and Polish Red breeds.

Frequency of genes controlling antigenic characters was calculated in the F, J, L, M, Z, N', R' and T' blood group systems (Table 2). Like in earlier studies with Black-and-White cattle (Trela et al., 1982; Rychlik, 1986), j, l, z, m, n,' t' genes were more frequent than J, L, M, Z, N', T' genes, whereas the F gene was more frequent than V and the S' gene more frequent than R'. The state of genetic equilibrium in the investigated population of Whitebacked cattle was evaluated based on the F and R' systems. In both systems, there was a discrepancy between the observed and expected number of genotypes. The disturbed genetic equilibrium can be attributed to the strict choice of bulls for further reproduction and the fact that the frequency of genes spread by a small number of bulls is higher than the frequency of genes in the cow population. The deviation from genetic equilibrium is also influenced by factors such as mating system, selection and migration.

The present findings provided extensive information on the polymorphism of erythrocyte antigens in 12 blood group systems of Whitebacked cattle from north-eastern Poland. Comparison of the frequency of some antigenic characters, the number and frequency of B-phenogroups and the degree of homozygosity with the value of these parameters calculated previously for Polish Red, Black-and-White and Red-and-White cattle revealed lower heterozygosity in this breed of cattle.

Because a certain amount of genetic variation is needed to obtain breeding progress, it is necessary to monitor changes taking place in the genetic structure of the population being improved. The present study, which in addition to the B system determined and calculated phenogroup frequency in the other complex systems (A, C and S) can serve as a starting point for further monitoring of genetic variation in this small population of cattle.

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Struktura genetyczna populacji rezerwy genetycznej bydła białogrzbietego na podstawie polimorfizmu grup krwi

STRESZCZENIE

Przy użyciu 75 standaryzowanych reagentów testowych określono skład antygenowy krwi u 92 sztuk bydła białogrzbietego z obszaru północno-wschodniej Polski. W układach grupowych krwi A, B, C, S obliczono częstość występowania ustalonych fenogrup, stopień homozygotyczności oraz efektywną liczbę alleli. W układzie B zidentyfikowano 29 fenogrup, wśród których z najwyższą częstością występowały: I2Q" (0,1849), G2Y2E'1Q'D" (0,1631) i E'3G"1Q" (0,0978), i Q" (0,0978). Wartość stopnia homozygotyczności w tym układzie wynosiła 9,14%, a efektywna liczba alleli 10,9. Przeprowadzona w układach F i R' analiza rozkładu genotypów wykazała istotne różnice między oczekiwaną i obserwowaną ilością genotypów, wskazującą na zachwianie równowagi genetycznej. W porównaniu do wcześniejszych badań struktury genetycznej innych ras objętych obecnie programem ochrony zasobów genetycznych, u bydła białogrzbietego stwierdzono wyższy stopień homozygotyczności i znacznie mniejszą liczbę fenogrup w układach grupowych krwi B i C, co wskazuje na mniejszą zmienność genetyczną w badanej populacji bydła białogrzbietego.